

Protective Effect of Saikosaponin D against Streptozotocin-Induced Diabetic Nephropathy in Rats by Regulating the Oxidative Stress and Inflammatory Markers

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ABSTRACT

Background: Diabetic Nephropathy (DN) is a common problem in diabetes and is characterized by glomerular dysfunction, hyperfiltration, and ultimately kidney failure. **Objectives:** The present study was intended to understand the salutary activities of Saikosaponin D in the Streptozotocin (STZ)-induced DN model. **Materials and Methods:** 60 mg/kg of STZ was administered to the rats to initiate DN, and they were then treated with Saikosaponin D (10 and 20 mg/kg, respectively) for 10 weeks. Insulin and blood glucose levels were measured using kits. The levels of renal function markers creatinine, urinary protein, Blood Urea Nitrogen (BUN), inflammatory cytokines, Malondialdehyde (MDA), antioxidant enzyme Catalase (CAT), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) activities were analyzed by the corresponding assay kits. A histopathological analysis of kidney tissues was conducted on both control and treated rats. **Results:** The Saikosaponin D treatment remarkably reduced blood glucose while boosting body weight and insulin in the DN rats. Creatinine, urinary protein, and BUN levels were considerably reduced by Saikosaponin D. It also diminished pro-inflammatory mediators and MDA levels while boosting antioxidant enzymes. The results of the histopathological analysis also revealed that Saikosaponin D considerably ameliorated STZ-induced histological damage in kidney tissues. **Conclusion:** The current findings of this study demonstrate the therapeutic properties of Saikosaponin D in mitigating the development of DN in STZ-induced rats. Therefore, it was clear that Saikosaponin D could be a promising candidate to treat diabetic nephropathy.

Keywords: Diabetic nephropathy, Saikosaponin D, Oxidative stress, Creatinine, Insulin.

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Received: 02-09-2023;

Revised: 11-12-2023;

Accepted: 09-03-2024.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic endocrine disease characterized by elevated blood glucose levels and poor carbohydrate, protein, and lipid metabolism. Type-I DM is caused by insufficient insulin secretion by the β -cells of the pancreas, while type-II DM is developed due to defective insulin function or insulin resistance. It was predicted that by 2045, around 700 million individuals around the world would have DM.¹ The hallmarks of DM include hyperglycemia, hypertension, dyslipidemia, microalbuminuria, and inflammation. Diabetic Nephropathy (DN) is a common condition that causes chronic and permanent damage to the small blood vessels and function of the kidneys in diabetic patients. The characteristics of the DN are glomerular dysfunction, hyperfiltration, and albuminuria.^{2,3}

In advanced disease conditions, proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis become prominent hallmarks. However, the exact mechanisms involved in this complex process have not been well elucidated. The various causes, including oxidative stress, glucose, advanced glycation end products, glomerular hypertension, and various signal transduction pathways, have been shown to play pivotal roles in the onset and development of DN.

The pathophysiology and progression of renal injury in diabetes are profoundly influenced by inflammation and oxidative stress. Hyperglycemia-induced oxidative and inflammatory responses are major contributors to the onset and development of DN; however, the precise molecular processes involved remain poorly understood at present. Oxidative stress causes enzyme inactivation, alterations in antioxidant systems, and cell membrane damage. Renal failure due to elevated oxidative stress leads to higher albumin excretion and a breakdown of the glomerular wall.⁴ DN has been connected with inflammation in the kidneys caused by a hyperglycemic condition. In diabetic kidneys, the inflammatory cascades are accelerated by a series



DOI: 10.5530/ijper.58.3.96

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of interconnected events, such as the local production of chemotactic proteins, an increased immune cell influx, and the following pro-inflammatory cytokine release. The pathophysiology of DN is significantly impacted by pro-inflammatory mediators.⁵ It was already suggested that Interleukin (IL)-6 activation led to abnormalities in the permeability of the glomerular endothelium, but the precise mechanisms concerning these cytokines have not yet been completely characterized. Moreover, IL-1 β has been associated with the DN progression. When TNF- α is over-expressed, it promotes the production of ROS and causes oxidative injury in the renal tissues.⁶

Despite numerous studies, DN is still the primary cause of mortality and disability among people with diabetes worldwide, making it a major public health concern. Moreover, there is still a lack of well-defined methods for treating DN.⁷ The existing therapies for DN include tight hyperglycemic control and the administration of antihypertensive and lipid-lowering medications, but these strategies are insufficient to prevent the development of DN in many patients. DN patients often need kidney transplantation or dialysis to restore renal function.⁸ Diabetes and its associated problems and comorbidities have a significant effect on healthcare expenditures.⁹ Consequently, the development of novel, safe medicines is of paramount importance for the treatment of DN patients.

Saikosaponin D is a major triterpenoid saponin compound that is obtained from the *Bupleurum falcatum* L. (Apiaceae) plant. Several studies have already shown that Saikosaponin D has therapeutic effects, such as anti-inflammatory,¹⁰ anti-cancer,¹¹ anti-ulcerative colitis,¹² anti-autoimmune disease,¹³ anti-liver fibrosis,¹⁴ anti-depressant,¹⁵ and anti-hyperlipidemic¹⁶ properties. Recent studies have highlighted that Saikosaponin D has therapeutic effects against diabetic peripheral neuropathy.¹⁷ Hence, we hypothesized that Saikosaponin D could be beneficial in ameliorating the DN. Thus, the current work was intended to understand the salutary activities of Saikosaponin D against STZ-induced DN in rats.

MATERIALS AND METHODS

Chemicals

The following chemicals and reagents were procured from Sigma Aldrich, USA: Saikosaponin D, STZ, etc. The assay kits for biochemical assays were procured from Thermo Fisher Scientific, USA.

Experimental animals

The 8-week-old male Sprague-Dawley rats were utilized in the current study. The rats used in the experiments were imprisoned in polypropylene confines and kept in a clean, controlled environment. Temperature 22-25°C, humidity between 40-70%, and a 12 hr light/dark sequence were kept consistent during the

housing of animals. All of the rats were given standard pellet food throughout the study. This study was approved by the Ethics Committee of Xingtai Central Hospital (approval number: 2023-KY-14).

Induction of DN and treatment protocols

DN was induced by giving 60 mg/kg STZ (in 0.1 M sodium citrate buffer). Blood glucose oxidase was measured 7 days after injection using kits (Thermo Fisher Scientific, USA), and concentrations greater than 16.7 mM were considered diabetic. After fourteen days of STZ injection, the diabetic rats were divided into three groups (Groups II-IV), each with six rats ($n=6$). The rats in Group I was non-diabetic and were only given citrate buffer without STZ. Group II rats served as diabetic controls, while Group III and IV rats were given Saikosaponin D (10 and 20 mg/kg, respectively). The Saikosaponin D treatment was administered via oral gavage once a day for ten weeks.

Analysis of blood glucose, body weight, glycosylated Hemoglobin (HbA_{1c}), and insulin levels in the experimental rats

The body weight of the experimental rats was measured frequently every week during the experiments. The status of HbA_{1c}, blood glucose, and insulin was determined using the assay kits according to the manufacturer's instructions (Thermofisher, USA).

Assay of urinary protein, Blood Urea Nitrogen (BUN), and creatinine levels in the experimental rats

The levels of BUN and creatinine in the serum, and protein in the urine, were analyzed using assay kits as per the manufacturer's instructions (Thermofisher, USA). Each assay was conducted in triplicates.

Analysis of pro-inflammatory cytokine levels

The corresponding assay kits were procured from Thermo Fisher Scientific, USA, and utilized to determine the status of TNF- α , IL-1 β , and IL-6 in the serum samples of experimental rats as per the instructions given by the manufacturer. Each assay was conducted in triplicate.

Analysis of oxidative stress markers in the renal tissues

The kidney tissue homogenates were prepared and subjected to measure the oxidative stress biomarkers level. To measure the status of lipid peroxidation, Malondialdehyde (MDA) was determined in the kidney tissue homogenates using a kit (Thermofisher, USA). The Glutathione Peroxidase (GPx), Catalase (CAT), and Superoxide Dismutase (SOD) activities in the kidney tissues of control and treated rats were determined by the kits using the manufacturer's instructions (Thermofisher, USA).

Histopathological analysis

Renal tissues were removed from both experimental rats, processed with formalin (10%), and embedded in paraffin wax. Subsequently, tissues were sectioned at 5 μm diameter, and hematoxylin-eosin (H and E) solution was employed for staining the sections to examine microscopically. Then, an optical microscope was employed to investigate the histological damage in the kidney tissues.

Statistical analysis

The statistical measurements were done using SPSS software. The data were scrutinized by one-way ANOVA and Duncan's Multiple Range Test (DMRT). The final data are revealed as a mean \pm SD of triplicate measurements with $p < 0.05$ as significant.

RESULTS

Effect of Saikosaponin D on the body weight, glucose, HbA_{1c} and insulin levels in the experimental rats

The changes in body weight, glucose, HbA_{1c}, and serum insulin levels in the control and Saikosaponin D-treated DN rats were analyzed, and the results are revealed in Figure 1. The blood glucose and HbA_{1c} were drastically increased, while the body weight and insulin were reduced in the DN rats. However, 10 and 20 mg/kg of Saikosaponin D fascinatingly reduced glucose and HbA_{1c} status and also boosted insulin and bodyweight levels in DN rats. These findings evidence the therapeutic properties of Saikosaponin D in the STZ-induced diabetic condition.

Effect of Saikosaponin D on the BUN, creatinine, and urine protein levels in the experimental rats

The influence of Saikosaponin D on kidney function biomarkers was analyzed, and the findings are shown in Figure 2. The increased BUN and creatinine status in the serum and protein in the urine were noted in the STZ-induced DN rats. Whereas, the creatinine, BUN, and urinary protein levels were effectively diminished by the Saikosaponin D in the DN rats. These outcomes evidence that Saikosaponin D considerably reduced the excretory product level and modulated renal functions in the rats with DN.

Effect of Saikosaponin D on the pro-inflammatory Cytokines in experimental rats

The status of pro-inflammatory cytokines in the serum of experimental rats was examined, and the findings are depicted in Figure 3. The findings exhibited that the DN rats had drastic elevations in the IL-6, TNF- α , and IL-1 β status in the serum when compared to the control. Interestingly, the Saikosaponin D treatment at concentrations of 10 and 20 mg/kg considerably diminished these cytokine levels in the DN rats. These results proved the anti-inflammatory activities of Saikosaponin D.

Effect of Saikosaponin D on the oxidative stress markers in the kidney tissues of the experimental rats

Figure 4 exhibits the results of an analysis of oxidative stress biomarkers in the renal tissues of the experimental rats. A drastic elevation in MDA level was noted, and a diminution in the GPx, SOD, and CAT activities was observed in the renal tissues of the DN rats. Fascinatingly, the Saikosaponin D treatment considerably reduced the MDA level while boosting the antioxidant enzyme activities in the kidney tissues of DN rats. The findings of these assays are evidence that Saikosaponin D has antioxidant properties.

Effect of Saikosaponin D on the kidney Histopathology the experimental Rats

Figure 5 depicts the results of a histological study of the kidney tissues of experimental rats. The renal tissues from control rats exhibited normal and intact histological arrangements with normal glomerular and corpuscular capillaries and distal and proximal convoluted tubules. Contrastingly, the kidney tissues of STZ-induced DN rats showed glomerulosclerosis, degeneration of tubules, lamina dilation, tubule epithelial lining atrophy, and hypertrophy of the tubule epithelial lining when compared to control. Interestingly, Saikosaponin D successfully ameliorated the histopathological damage in the kidney tissues of the DN rats (Figure 5).

DISCUSSION

Diabetic patients frequently develop chronic kidney disease as a result of pathological renal microvascular alterations. Hyperglycemia increases the abnormal protein kinase activation, inflammation, oxidative stress, and extracellular matrix generation, which causes DM-associated metabolic changes and kidney damage.¹⁸ Hyperglycemia directly, causes an increase in glomerular filtration and tubular workload by stimulating the kidneys to filter and reabsorb glucose for optimal kidney function.¹⁹ In DM patients, DN is a major cause of morbidity and even death. Nonetheless, there is still a challenge in treating DN, and the use of antidiabetic medicines is limited by several serious side effects. Thus, the current work was intended to disclose the therapeutic potential of Saikosaponin D on rats with DN.

STZ has been extensively utilized to induce the experimental DM model in animals because of its potential to cause degranulation and loss of insulin-secreting capability in pancreatic beta cells.²⁰ Therefore, in this work, STZ was employed to induce the DN model in rats. STZ-induced DM is linked to a considerable reduction in body weight as a result of hyperglycemia, enhanced muscle degeneration, and tissue protein loss.²¹ Similarly the current findings also noted that the DN rats exhibited a considerable decrease in body weight. Whereas, the Saikosaponin D treatment fascinatingly increased the body weight of the DN rats.

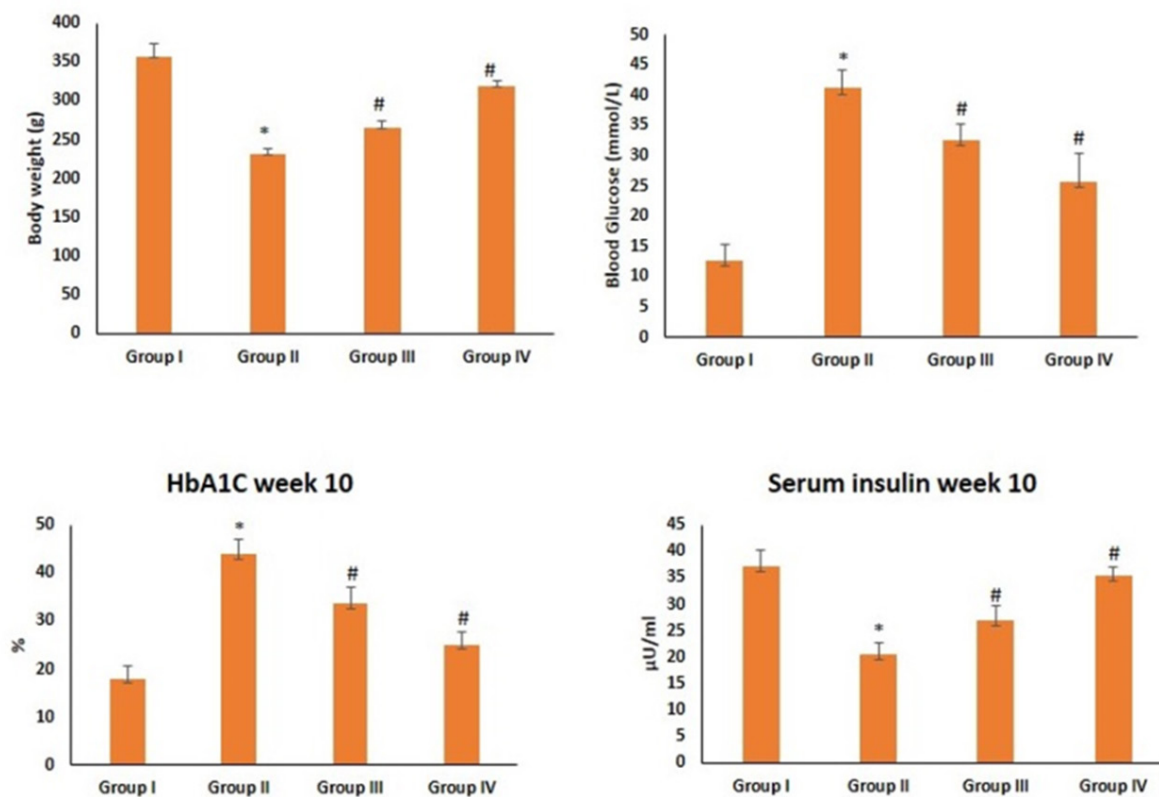


Figure 1: The Saikosaponin D treatment considerably decreased blood glucose and HbA_{1c} while increasing body weight and insulin in the rats with DN. Values are indicated as a mean±SD of values that were obtained from triplicate assays. Data were measured by one-way ANOVA and DMRT assay using SPSS software. Note: '*' represents that data were significant at $p < 0.01$ from the control and '#' indicates that values were significant at $p < 0.05$ from the STZ-induced DN group.

Inflammation is an imperative factor in DN development. The production of cytokines and inflammatory regulators increases during an inflammatory response. Hyperglycemia and inflammation cause the IL-1 β , IL-6, and TNF- α release, which further aggravate DN development.²² TNF- α is a powerful cytokine and a pivotal player in the onset of DN and other microvascular diabetic problems. TNF- α is highly cytotoxic to epithelial, glomerular, and mesangial cells. The free radicals produced by TNF- α also contribute directly to renal damage.²³ Increased levels of IL-6 also speed up mesangial cell growth, increase fibronectin, and cause changes in extracellular matrix dynamics.²⁴ IL-1 β is a prostaglandin synthesis-related cytokine that has been linked to the onset of abnormalities in intraglomerular hemodynamics.²⁵ Overexpression of specific cytokines in the renal tissues and an elevated inflow of circulating immune cells have both been observed in numerous studies on the diabetic model.^{26,27} Furthermore, inhibiting these cytokine productions therapeutically is thought to hinder glomerular and tubulointerstitial damage in DN patients, indicating their relevance as prospective therapeutic targets.²⁸ In line with these statements, the current findings also revealed elevated inflammatory cytokine levels in the DN rats. Interestingly, the treatment with Saikosaponin D remarkably diminished

these cytokine levels. These outcomes are evidencing the anti-inflammatory potential of Saikosaponin D.

Oxidative stress is characterized by the uncontrolled generation of free radicals and is caused by persistent hyperglycemia. The kidneys are one of the main organs that are especially vulnerable to oxidative injury.²⁹ Oxidative stress is increasingly recognized as a crucial contributor to several diseases, including DN.³⁰ The increased oxidative stress can aggravate renal inflammation, apoptosis, and fibrosis, thereby playing a critical role in the DN progression.³¹ Oxidative stress has been thought to accelerate the onset and development of DN via multiple processes,³² including a surge in glomerular hyperfiltration and the direct induction of renal cell destruction. Hyperglycemia increases the generation of ROS, which are involved in the pathogenesis of numerous diabetes comorbidities.³³ The ROS diminishes the antioxidant defenses, which makes it more vulnerable to oxidative injury. It then targets lipids, DNA, and proteins, causing them to oxidize, resulting in cellular damage.

MDA, a by product of lipid oxidation, is a key biomarker of lipid peroxidation. Cellular oxidative stress is indicated by changes in MDA and antioxidants, including GPx, SOD, and CAT levels. The CAT enzyme degrades superoxide radicals, which are then reduced to water by SOD. As a result, CAT aids SOD

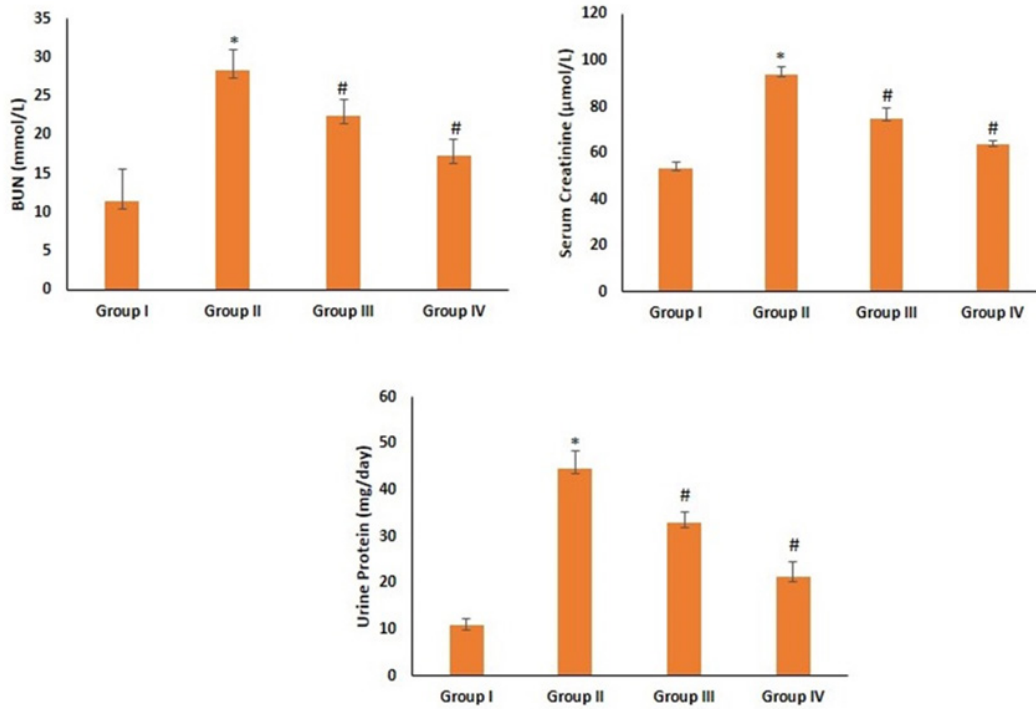


Figure 2: The creatinine, urinary protein, and BUN levels were effectively decreased in the DN rats by the Saikosaponin D treatment. Values are indicated as a mean±SD of values that were obtained from triplicate assays. Data were measured by one-way ANOVA and DMRT assay using SPSS software. Note: '*' represents that data were significant at $p < 0.01$ from the control and '#' indicates that values were significant at $p < 0.05$ from the STZ-induced DN group.

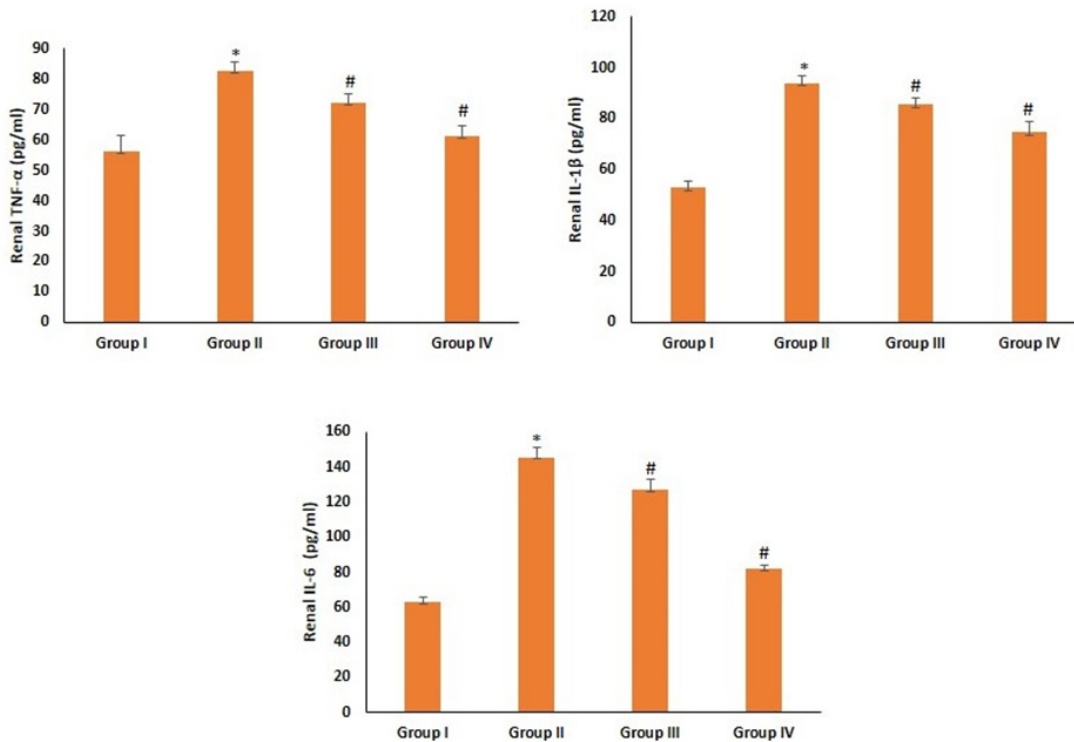


Figure 3: The treatment with Saikosaponin D remarkably reduced the IL-6, TNF-α, and IL-1β in the DN rats. Values are indicated as a mean±SD of values that were obtained from triplicate assays. Data were measured by one-way ANOVA and DMRT assay using SPSS software. Note: '*' represents that data were significant at $p < 0.01$ from the control and '#' indicates that values were significant at $p < 0.05$ from the STZ-induced DN group.

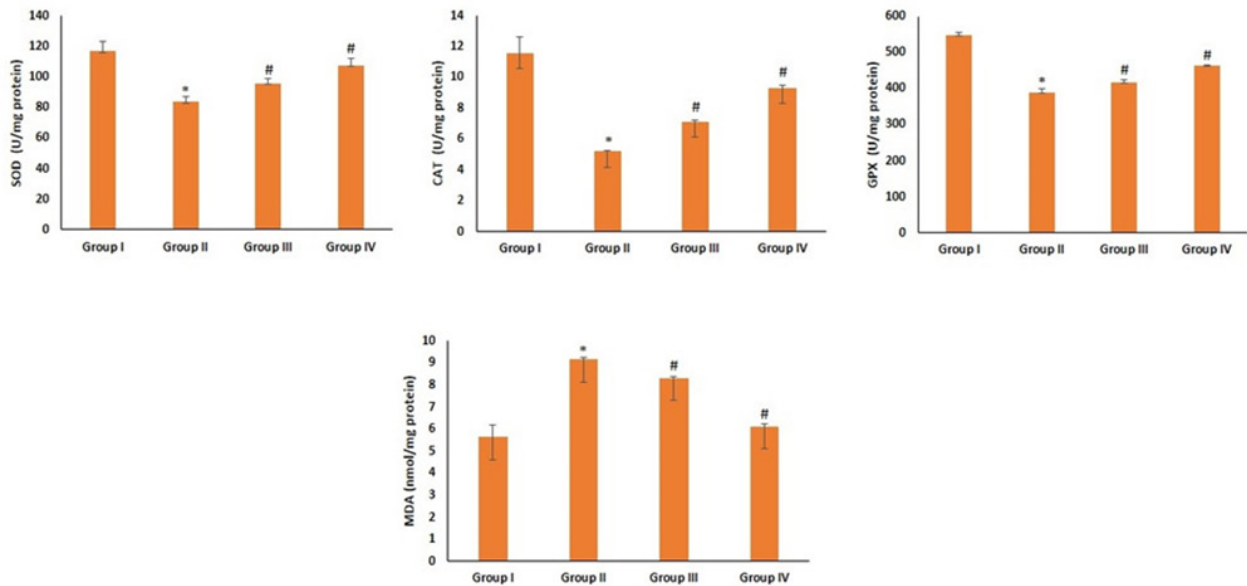


Figure 4: The findings proved that the Saikosaponin D treatment reduced the MDA level while boosting the antioxidant enzyme (CAT, SOD, and GPx) activities in the DN rats. Values are indicated as a mean±SD of values that were obtained from triplicate assays. Data were measured by one-way ANOVA and DMRT assay using SPSS software. Note: '*' represents that data were significant at $p < 0.01$ from the control and '#' indicates that values were significant at $p < 0.05$ from the STZ-induced DN group.

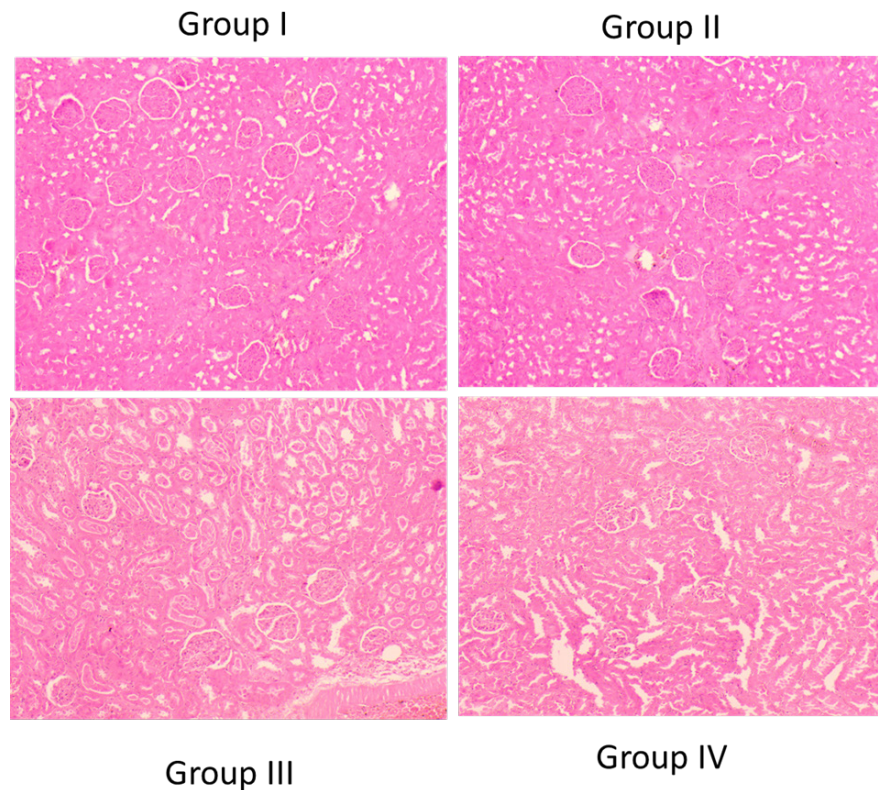


Figure 5: Group I: The kidney tissues from the Group I control rats demonstrated normal and intact histological arrangements with normal glomerular and corpuscle capillaries and distal and proximal convoluted tubules. Group II: The kidney tissues of STZ-induced DN rats showed glomerulo sclerosis, tubular degeneration, interstitial fibrosis, and hypertrophy of the tubule epithelial lining. Groups III and IV: The Saikosaponin D (10 and 20 mg/kg, respectively) considerably attenuated the histopathological damage in the kidney tissues of the DN rats.

in completely neutralizing ROS.³⁴ Several studies have shown that STZ-induced DN causes an augmentation in MDA and a diminution in GSH, SOD, and CAT.³⁵⁻³⁷ In this study, our results exhibited a severe increase in the MDA level and a diminution in the antioxidant enzyme activities in the kidney tissues of the DN rats. Interestingly, the Saikosaponin D treatment considerably reduced the MDA and boosted the antioxidants in the DN rats. These results corroborate the antioxidant properties of Saikosaponin D in the STZ-induced DN model.

Diabetic patients often have elevated levels of serum and urinary proteins and other excretory products such as urea, creatinine, and BUN.³⁸ The serum creatinine and BUN levels are usually higher in the diabetic condition than in the non-diabetic milieu. The glomerulus of a diabetic control showed serious pathological alterations, including the loss of glomerulus tuft and capillaries and the widening of Bowman's space, both of which are crucial to glomerular filtration.³⁹ The impaired glomerular filtration may lead to an elevation of serum creatinine levels. In addition, kidney tissue from untreated diabetic rats showed damage to the tubules, most noticeably in the proximal tubules. Most of the filtrates, including creatinine, are reabsorbed in the proximal tubule, making it a critical organ in the reabsorption process. Serum creatinine levels were probably high because of kidney injury, specifically to the glomeruli and tubules.⁴⁰ Progressive kidney abnormalities cause proteinuria by disrupting renal hemodynamics.⁴¹

Proteinuria is a complication of intraglomerular hypertension, which causes glomerular sclerosis, podocyte destruction, and disruption of the basement membrane.⁴² The increased amounts of urine protein are the result of renal tissue injury in response to the DN-related release of pro-inflammatory factors, which hastens the invasion of macrophages. Here, in DN rats, BUN and creatinine in the serum and protein in the urine are found in higher concentrations when compared to the control. Whereas, the Saikosaponin D treatment substantially decreased these markers in the STZ-induced DN rats. These outcomes prove that the Saikosaponin D treatment promotes renal function in DN rats. The findings of the histopathological analysis also revealed that Saikosaponin D effectively attenuated the histopathological damage in the kidney tissues of the DN rats, evidencing the therapeutic potential of Saikosaponin D.

CONCLUSION

The findings of the current work demonstrated the therapeutic activity of Saikosaponin D in attenuating the progression of DN in the rats. The Saikosaponin D treatment remarkably decreased the blood glucose, increased insulin levels, averted inflammation and oxidative stress via boosting antioxidants, and attenuated kidney damage in the rats with DN. Therefore, it was clear that Saikosaponin D could be a talented salutary candidate to treat the DN. In addition, more experiments in this context are highly

recommended in the future to comprehend the underlying therapeutic activities of Saikosaponin D against DN.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DN: Diabetic nephropathy; **STZ:** Streptozotocin; **BUN:** Blood urea nitrogen; **MDA:** Malondialdehyde; **CAT:** Catalase; **SOD:** Superoxide dismutase; **GPx:** Glutathione peroxidase; **DM:** Diabetes mellitus; **IL-6:** Intereukin-6; **H and E:** Hematoxylin-Eosin.

SUMMARY

Diabetic patients frequently develop chronic kidney disease as a result of pathological renal microvascular alterations. Hyperglycemia increases the abnormal protein kinase activation, inflammation, oxidative stress, and extracellular matrix generation, which causes Diabetes Mellitus (DM), associated metabolic changes, and kidney damage. Diabetic Nephropathy (DN) is a common problem of diabetes and is characterized by glomerular dysfunction, hyperfiltration, and ultimately kidney failure. DN is a major cause of morbidity and even death. Nonetheless, there is still a challenge in treating DN, and the use of antidiabetic medicines is limited by several serious side effects. Consequently, the development of novel, safe medicines is of paramount importance for the treatment of DN patients. Saikosaponin D is a major triterpenoid saponin compound that is obtained from the *Bupleurum falcatum* L. (Apiaceae) plant. Several studies have already shown that Saikosaponin D has therapeutic effects, such as anti-inflammatory, and anti-cancer. Recent studies have highlighted that Saikosaponin D has therapeutic effects against diabetic peripheral neuropathy. The present study was intended to understand the salutary activities of Saikosaponin D in the Streptozotocin (STZ)-induced DN model. The levels of renal function markers, blood urea nitrogen, antioxidant enzymes, inflammatory cytokines, and histopathological analysis. The current findings of this work reveal the therapeutic properties of Saikosaponin D in attenuating the progression of DN in STZ-induced rats. Therefore, it was clear that Saikosaponin D can be a promising salutary candidate to treat the DN

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Cite this article: Chen Y, Zhang G, Liang R, Shen Y, Jiao X. Protective Effect of Saikosaponin D against Streptozotocin-Induced Diabetic Nephropathy in Rats by Regulating the Oxidative Stress and Inflammatory Markers. *Indian J of Pharmaceutical Education and Research.* 2024;58(3):882-9.